Original article

The pore in the hard conical *Apis cerana* drone capping results from a spinning process

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Abstract – A central, funnel-like pore in the conical cap (measuring 0.3 mm in depth and 0.25–0.50 mm in diameter) is typical for *Apis cerana* drone brood cells. Scanning electron microscopy was used to examine the spinning process and the origin of the pore from dated *A. cerana* drone brood cells. In contrast to earlier reports the pore in *A. cerana* drone cell caps is constructed by rotating movements of the drone larva during the spinning process. Unresolved questions concerning the biological significance of the pore are discussed. The reason why this hard conical cap with the central, funnel-like pore evolved only in *A. cerana* and in *A. koschevnikovi*, however, remains uncertain. © Inra/DIB/AGIB/Elsevier, Paris

Apis cerana / cocoon / drone

1. INTRODUCTION

Apis cerana drone larvae spin a cocoon shortly before pupation, just as workers, queens and drones of the other eight honey bee species. This is typical for holometabolic insects. Cocoons consist mainly of silk gland secretion [5]. A central, funnel-like pore in

the conical cap (measuring 0.3 mm in depth and 0.25–0.50 mm in diameter) is typical for *A. cerana* drone brood cells (see *figure 1*) [3, 4, 13–15]. In *A. koschevnikovi*, a species closely related to *A. cerana*, capped drone brood cells likewise have a pore [1, 6], but this unique structure is absent in all the other honeybee species. In *A. nigrocincta*, which

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Figure 1. The final stage of an *Apis cerana* drone cocoon cap. Thirty hours after the cell was sealed the outer wax capping (A) is removed together with the spongy cocoon material (B) by the worker bees, exposing the hard cap and the central pore.

is also related to *A. cerana*, even the hard conical cap is absent [3].

The pore in the cocoon cap of *A. cerana* drone brood cells usually appears within a few days following pupation, after worker bees remove the wax sealing. Hänel and Ruttner [4] presumed that the central pore results from a local enzymatic dissolution of the cocoon material from the inside after completion of the whole cocoon cap.

Some doubt about the action of a drone larval secretion has emerged after direct observations in *A. cerana* colonies in Japan, northern India, the Philippines and Thailand. In order to examine the origin of the pore in the drone cell cap, cocoon material was collected 1985 in Tokyo (Japan), 1991 in Allahabad (northern India), 1992 in Chiang Mai (Thailand) and 1996 in Bacnotan, La Union (the Philippines).

2. MATERIALS AND METHODS

The capping time of individual *A. cerana* drone brood cells was recorded (at 4-h intervals) and their position in the brood area marked on

transparent sheets temporarily attached to the top bar of the frame. The frames were returned to their hives between observations. In intervals of 6 h following the capping single drone cell caps were carefully removed using a razor blade in order to investigate them later using scanning electron microscopy (SEM).

3. RESULTS

A. cerana drones start to spin threads of silk against the thin beeswax layer constructed by the workers during sealing. At this early stage the cocoon is of spongy consistency in contrast to the next layers which are hard and of dense structure (figure 2, cross-section). One or a few days after the cell is sealed, the wax sealing is removed together with the spongy cocoon material by the worker bees until the hard cap becomes visible (figure 1).

The sequences of SEM pictures (inside view, figures 3–5) show that *A. cerana* drones construct the pore while spinning the cocoon cap. Even early in this process the position and the basic shape of a pore is visible from the inside view (here regis-

Figure 2. Cross-section of an *Apis cerana* drone cocoon cap after completion (30 h after the sealing of the cell). The outside part of the cocoon is of a spongy consistency (B) in contrast to the subsequent layers which are hard and of dense structure (C). The molten-like wall texture inside the pore (D) is most probably formed during the spinning, when the secreted silk material is still in a liquid state before solidifying.

Figure 3. Already at the very beginning of spinning the position and basic shape of the pore is visible from the inside view (here registered 6 h after sealing of the cell).

tered 6 h after capping, *figure 3*). However, if the thin wax layer and the spongy cocoon material are experimentally removed, the pore can be seen even from the outside at this early stage. The central pore takes on a funnel-like shape as the spinning proceeds. The basic elements of cocoon construction

are thin threads and sheets of silk material (*figure 4*). The course of single cocoon threads around the pore (*figure 3*) imply rotating movements of the drone larva during the spinning of the cocoon cap. Inside the channel of the pore the basic elements of cocoon construction are thin threads and

Figure 4. The central pore takes on a funnel-like shape as the spinning proceeds (inside view registered 12 h after sealing of the cell). The basic elements of cocoon construction are thin threads and sheets of silk material.

sheets of silk which are sometimes glued together (figure 5). This is similar for *A. cerana* drone cell caps from Japan, northern India, Thailand and the Philippines.

4. DISCUSSION

SEM was used to examine the spinning process and the origin of the pore from dated *A. cerana* drone brood cells. This study demonstrated that the pore in *A. cerana* drone cell caps is constructed during the spinning process. During cocoon construction the predominant movement made by larvae (drone, worker and queen) of *A. mellifera* is a forward somersault [5]. However, the course of single cocoon threads around the pore imply intensive rotating movements of the *A. cerana* drone larva during spinning of the cocoon cap. This is in contrast to the report by Hänel and Ruttner [4] who presumed that the central pore results from a local enzymatic dissolution of the cocoon material. They assumed that the drone larvae might secrete a cocoon-dissolving enzyme after completion of the whole cocoon cap, as is known from *Lepidoptera*.

In cocoons that are completed the basic elements of cocoon construction inside the channel of the pore are thin threads and sheets of silk, which are sometimes glued together. A dark brown substance is deposited along the inner surface of the pore, which is thicker near the inner passage of the pore and is thinned out towards the periphery [15]. Enzymatic dissolution presumed by Hänel and Ruttner [4] seemed likely from the wall surface texture inside the pore of completed cocoons (figure 2, cross-section, D). However, these molten-like structures are most probably formed during the spinning, when the secreted silk material is still in a liquid state before solidifying. If one looks at the inner surface of the cocoon of the silk moth, *Antheraea pernyi* or *Bombyx mori*, to which cocoonase is never applied, a very similar structure exists (Sasaki, pers. obs.). Silk moth cocoonase is secreted shortly before the adult moth emerges. The hole formation is accomplished by the change or rearrangement of fibroin fibres as a result of dissolution of sericin (glue material) and active and repetitive pushing behaviour towards the moist-

Figure 5. Inside the channel of the pore the basic elements of cocoon construction are thin threads and sheets of silk which are sometimes glued together.

ened position by the head of the adult moth. We could not clarify the surface texture inside the pores of completed cocoons of *A. cerana*, especially the dark brown substance which is deposited along the inner surface. Possibly it is faeces discharged from the larval anus after the completion of the cocoon [5].

The pore in the cap obviously serves for the exchange of respiration gases by the developing drone in the sealed cell. This biological function was proven by plugging the cocoon pore experimentally with beeswax [9]. Drones did not emerge from cells when the central pore was closed in this way. Their metamorphosis was stopped or retarded. The dense cocoon structure obviously hinders the exchange of respiration gases. It is striking that if brood cells of *A. cerana* are experimentally uncapped, those of workers are re-sealed. When drone cells are uncapped, the drone pupae are fast removed by eating [9]. In *A. mellifera* both drone and worker brood are re-sealed if experimentally uncapped. In one experiment 94.9 ± 2.3 % of the worker cells ($n = 875$) were re-sealed and likewise 94.6

± 4.4 % of the drone cells ($n = 821$) using different developmental stages of the pupae (Boecking, unpublished data). A biological explanation for these species-specific differences is difficult. The dense and hard cocoon cap structure could protect the *A. cerana* drone pupae against cannibalism behaviour of the nurse bees, probably as a consequence of the queen-worker conflict on the optimal sex ratio of the brood (see Moritz and Southwick, chapter 6 [7]). But this does not explain why such a behaviour occurs in *A. cerana* alone. Another hypothesis is related to the *V. jacobsoni*-specific hygienic behaviour of the nurse bees. *A. cerana* worker bees are very effective in uncapping and removal of *V. jacobsoni*-infested worker brood [8, 10, 12]. In contrast, mite-infested sealed drone brood is removed to a very low extent. Also freeze-killed worker brood is removed quite fast compared to freeze-killed drone brood [10]. It is not clear if *A. cerana* bees avoid removing diseased or mite-infested drone brood, or if they simply are hindered by the hard cocoon cap [2]. Rath [9] assumed that this unique structure has possibly evolved under a male-biased

selection pressure in *A. cerana* against the *V. jacobsoni*-specific hygienic behaviour of the worker bees. But recent observations on a reduced fitness of *V. jacobsoni*-infested drones in *A. mellifera* (Rinderer et al. [11]) contradict these hypothesis.

Therefore, the biological significance of the hard conical cap with the central, funnel-like pore and the reason why this structure has evolved only in *A. cerana* and in *A. koschevnikovi* remains unsolved.

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Résumé – Le pore dans l'opercule dur et conique des cellules de mâles d'*Apis cerana* résulte d'un processus de filage.

La présence d'un pore central dans l'opercule dur et conique (environ 0,3 mm de profondeur et 0,25 à 0,5 mm de diamètre) est typique des cellules de couvain de mâles chez *Apis cerana*. Chez *A. koschevnikovi* aussi, les cellules de couvain de mâles possèdent cette structure de cocon unique, mais elle est absente chez toutes les autres espèces d'*Apis*, y compris chez *A. nigrocincta*, espèce proche de *A. cerana*.

Dans des publications antérieures il a été supposé que le pore central de l'opercule

était dû à une dissolution locale du cocon par les enzymes à la fin du processus de filage.

On a étudié au microscope électronique à balayage (MEB) le processus de filage et la formation du pore sur des opercules de cellules de mâles d'âge connu. Le moment de l'operculation des cellules a été marqué sur des feuilles transparentes qui ont été fixées à la baguette supérieure de chaque cadre. Entre les observations les rayons ont été remplacés dans leur ruche. À intervalles de 6 h après l'operculation, des opercules ont été prélevés avec soin à l'aide d'une lame de rasoir pour être étudiés ensuite au MEB.

L'étude montre que le pore dans l'opercule des cellules de couvain de mâles d'*A. cerana* se forme au cours du processus de filage. Les séries de photos au MEB (*figures 3 à 5*) prouvent que les mâles d'*A. cerana* ménagent le pore pendant le filage du cocon. Dès le début du processus de filage, la position et la forme de base du pore est visible de l'intérieur (*figure 3*, prise 6 h après l'operculation). Le trajet des fils du cocon autour du pore implique des mouvements de rotation de la larve mâle pendant le filage. La signification biologique du pore et la raison pour laquelle ce cocon dur et conique avec un pore central ne se rencontre que chez *A. cerana* et *A. nigrocincta* restent inexplicables. © Inra/DIB/AGIB/Elsevier, Paris

Apis cerana / cocon / mâle

Zusammenfassung – Die Pore im Deckel der Drohnenzelle von *Apis cerana* entsteht während des Einspinnens der Larve.

Typisch für *A. cerana* Drohnenbrutzellen ist die zentrale Pore im dicken, fest und konisch geformten Zellockel (die etwa 0,3 mm tief ist und einen Durchmesser von 0,25–0,5 mm besitzt). Auch *A. koschevnikovi* Drohnenbrutzellen sind mit dieser einzigartigen Kokonstruktur ausgestattet. Sie fehlt hingegen bei allen übrigen Honigbienenarten. Bei *A. nigrocincta*, die nah verwandt mit *A. cerana* sind, fehlt sogar der typisch dicke, feste Kokon.

In früheren Veröffentlichungen wurde angenommen, daß die zentrale Pore im *A. cerana* Drohnenzelldeckel nach Beendigung des Spinnvorgangs durch eine lokale enzymatische Auflösung des Kokonmaterials entsteht.

Mit Hilfe der Rasterelektronenmikroskopie REM wurde anhand von altersdefinierten Drohnenbrutzelldeckeln der Spinnprozess und die Entstehung der Pore untersucht. Dazu wurde zunächst der Verdecklungszeitpunkt einzelner Drohnenbrutzellen im zeitlichen Intervall von 4 Stunden erfaßt. Die Position dieser Zellen im Brutnest wurde auf transparenten Folien festgehalten, die dazu jeweils am Rähmchenoberträger fixiert wurden. Zwischen diesen Erfassungen befanden sich die Waben jeweils in ihrem Bienenvolk. Im zeitlichen Abstand von 6 Stunden, bezogen auf den Verdecklungszeitpunkt, erfolgte eine Entnahme einzelner Drohnenzelldeckel, die mit Hilfe einer Rasierklinge vorsichtig abgetrennt wurden. Später erfolgte eine Untersuchung mittels REM.

Die Untersuchungen zeigen, daß die Pore im *A. cerana* Drohnenzelldeckel schon während des Spinnvorgangs entsteht. Die Abfolge der REM-Aufnahmen (siehe *Abbildungen 3 bis 5*) belegen, daß *A. cerana* Drohnen während des Spinnens des Kokon die Pore aussparen. Schon zu Beginn des Spinnvorgangs ist die Position und die Grundgestalt der Pore von der Innenseite her zu erkennen (siehe *Abbildung 3*, hier 6 Stunden nach dem Verdecklungszeitpunkt erfaßt). Der Verlauf einzelner Kokonfäden um die Pore herum (*Abbildung 3*) läßt auf kreisende Bewegungen der Drohnenlarve während des Spinnvorgangs schließen.

Die biologische Bedeutung der Pore und der Grund, wofür dieser harte konische Kokon mit der zentralen Pore sich nur bei *A. cerana* und *A. koschevnikovi* entwickelte, bleibt jedoch ungeklärt. © Inra/DIB/AGIB/Elsevier, Paris

***Apis cerana* / Kokon / Spinndrüsen / Drohn**

REFERENCES

- [1] Alexander B.A., Phylogenetic analysis of the genus *Apis* (Hymenoptera: Apidae), *Ann. Entomol. Soc. Am.* 84 (1991) 137–149.
- [2] Boecking O., Non-removal and sealing up diseased and *Varroa* infested drone brood cells is part of the hygienic behavior in *Apis cerana* bees, *J. Apic. Res.* 38 (1999) (in press).
- [3] Hadisoelilo S., Otis G.W., Differences in drone cappings of *Apis cerana* and *Apis nigrocincta*, *J. Apic. Res.* 37 (1998) 11–15.
- [4] Hänel H., Ruttner F., The origin of the pore in the drone cell capping of *Apis cerana* Fabr., *Apidologie* 16 (1985) 157–164.
- [5] Jay S.C., The cocoon of the honeybee, *Apis mellifera* L., *Can. Entomol.* 96 (1964) 784–792.
- [6] Mathew K.P., Mathew S., Further observations on characters and behaviour of *Apis koschevnikovi*, the 'red bee' of Sabah, *Bee World* 71 (1990) 61–66.
- [7] Moritz R.F.A., Southwick E.E., Bees as Superorganisms, Springer-Verlag, Berlin, Heidelberg, NY, 395 p.
- [8] Peng Y.S., Fang Y., Xu S., Ge L., Nasr M.E., Response of foster Asian honeybee (*Apis cerana* Fabr.) colonies to the brood of European honeybee (*Apis mellifera* L.) infested with parasitic mite *Varroa jacobsoni* Oudemans, *J. Invertebr. Pathol.* 49 (1987) 259–264.
- [9] Rath W., The key to *Varroa*: The drones of *Apis cerana* and their cell cap, *Am. Bee J.* 132 (1992) 329–331.
- [10] Rath W., Drescher W., Response of *Apis cerana* Fabr. towards brood infested with *Varroa jacobsoni* Oud. and infestation rate of colonies in Thailand, *Apidologie* 21 (1990) 311–321.
- [11] Rinderer T.E., De Guzman L.I., Lancaster V.A., Delatte G.T., Stelzer J.A., *Varroa* in the mating yard: I. Effects of *Varroa jacobsoni* and *Apistan*® on drone honeybees, *Am. Bee J.* 139 (1999) 134–139.
- [12] Rosenkranz P., Tewarson N.C., Singh A., Engels W., Differential hygienic behaviour towards *Varroa jacobsoni* in capped worker brood of *Apis cerana* depends on alien scent adhering to the mites, *J. Apic. Res.* 32 (1993) 89–93.
- [13] Sakagami S.F., Preliminary report on the specific difference of behaviour and other ecological characters between European and Japanese honeybees, *Acta Hymenopterologica* 1 (1960) 171–198.
- [14] Sasaki M., Ono M., Yoshida T., Some biological aspects of the north-adapted eastern honeybee, *Apis cerana japonica*, in: Kevan P.G. (Ed.), *The Asiatic Hive Bee: Apiculture, Biology and Role in Sustainable Development in Tropical and Subtropical Asia*, Enviroquest Ltd, Cambridge, Ontario, Canada, 1995, pp. 59–78.
- [15] Tokuda, Y., Studies on the honeybee, with special reference to the Japanese honeybee, *Trans. Sapporo Nat. Hist. Soc.* 9 (1924) 1–27 (in Japanese).